

Extraction Efficacy of Synthetic Cannabinoids From Damiana Leaf Substrates Utilizing Electrolytic Solvents

by Abby L. West, Nabila Hoque, and Mark H. Griep

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14. ABSTRACT The study described in this report determined a rapid and facile method to extract synthetic cannabinoids from leafy substrates in the field. Win 55, 212-2 was used as the model synthetic cannabinoid while damiana leaf cuttings were used as a representative substrate material. Both low- (2%) and high- (100%) solvent concentrations were assayed for overall extraction efficiency and it was determined that extraction with pure solvent leads to a much higher yield of cannabinoid with ethanol extracting the largest quantity of cannabinoid. Interestingly, there is no significant difference between extraction times of 30 s and 1 min with hand shaking. Thus, it was determined that extraction of a small amount of substance (approximately 10 mg) with 100% ethanol and 30 s of shaking yields a sufficient amount of synthetic cannabinoid compounds for further analysis techniques. Further studies are needed to determine if these extraction parameters are compatible with both real-world “spice” samples and a wide range of synthetic cannabinoid compounds.					
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1. Introduction and Background

Herbal mixtures, such as “Spice” and “K2,” were exposed as hosts for synthetic molecules that imitate the effects of the psychoactive component of cannabis, delta-9-tetrahydrocannabinol (THC) (1–5). Synthetic cannabinoid receptor agonists comprise a diverse group of chemically unrelated substances that have long evaded the U.S. legal system by being sprayed onto an inert dry plant substrate and subsequently merchandized as “incense,” “plant food,” and “room odorizers” from as early as 2004 (1, 6–10). These herbal blends are also widely available on the internet and in retail outlets such as smoke and head shops with disclaimers that read “not for human consumption” with only natural ingredients listed on their packages (11). When further tested, they have been found to contain neither tobacco nor cannabis, but still produce cannabimimetic effects. As a result, these herbal mixtures doped with synthetic cannabinoids have become widely abused as a supposed legal alternative to cannabis (12–14).

Unfortunately, synthetic cannabinoids have also become a significant problem within the U.S. Armed Forces (10, 15–17). “Spice” and other related herbal products are readily abused by many military personnel as they can be purchased without age restrictions, are not detected in standard drug screens, and are commonly misinterpreted as safe since they are marketed as “herbal” and “natural” products (18, 19). Consequently, these new and harmful cannabis substitutes pose major public health and legal predicaments. Scheduling these compounds presents a unique concern as there are so many cannabimimetic compounds available (20, 21). Each time one synthetic cannabinoid is regulated, another derivative is made available that is not subject to regulation (9, 17, 22). Thus, the U.S. Drug Enforcement Administration (DEA) has moved toward scheduling the entire class of compounds. However, defining the class of compounds is problematic as the structures of these cannabimimetic compounds are very structurally diverse (figure 1) (23).

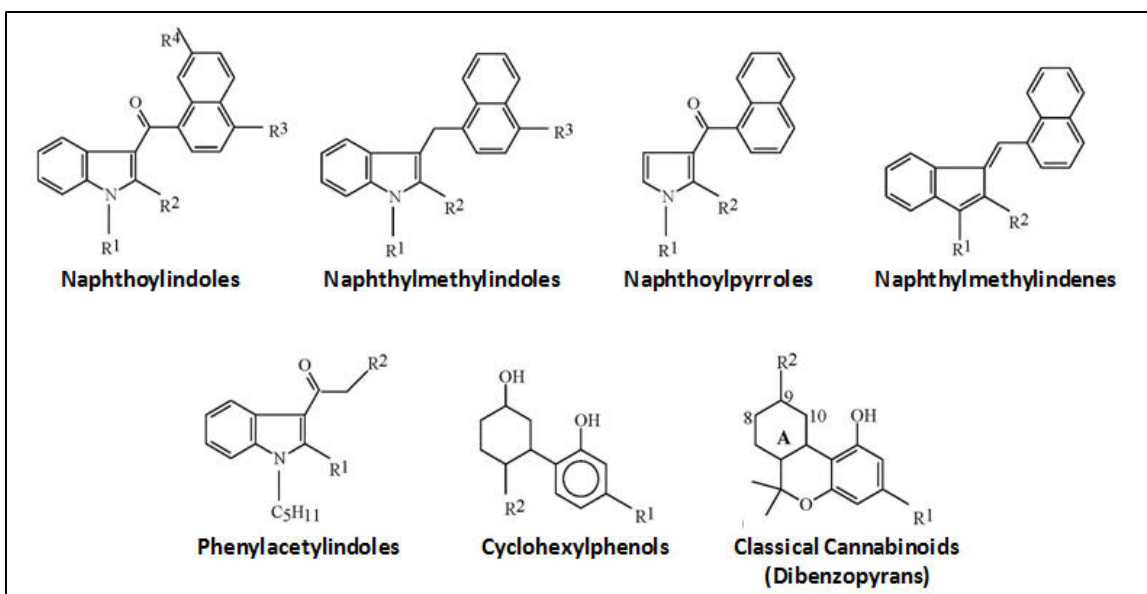


Figure 1. Basic chemical structures of the seven different synthetic cannabinoid groups.

Synthetic cannabinoids (SCs) elicit physiological responses comparable to THC by binding and activating the same cannabinoid receptors (CB) in the body, CB1 and CB2 (19, 24). CB1 is expressed in the central nervous system whereas CB2 is expressed in the peripheral nervous system (25). CB1 is primarily responsible for the psychological and physiological effects generated by SC binding to the receptors (26). SCs have been studied and developed for over 20 years with the original intent to be used for pharmacotherapeutic purposes; however, companies and researchers were never able to circumvent the negative psychoactive side effects (27). They also exhibit potencies from 10 to 100 times greater than that of THC, which raises many health concerns (6, 18, 19, 28–32). Synthetic cannabinoid receptor agonists are categorized into seven major groups: naphthoylindoles [n=74], naphthylmethylindoles [n=9], naphthoylpyrroles [n=32], naphthylmethylindenes [n=3], phenylacetylindoles (i.e., benzoylindoles) [n=28], cyclohexylphenols [n=16], and classical cannabinoids (dibenzopyrans) (figure 1) (33). The numbers in brackets are indicative of the amount of members in each group that activate the CB1 receptor and are therefore cannabimimetic. The numbers of known SCs increase almost daily, with new analogs being synthesized to continue to skirt the legal system.

After identifying substances like JWH-018 and CP-47,497 in spice products in 2008, they were banned in many European countries. The National Forensics Laboratory of the DEA reported in 2011 that the number of synthetic cannabinoid samples submitted to forensic laboratories rose from 13 to a staggering 2977 cases between 2009 and 2010. As the current primary means of detection is Gas Chromatography Coupled Mass Spectrometry (GC-MS) and Liquid Chromatography Coupled Mass Spectrometry (LC-MS) technologies, it is necessary to optimize the extraction method for isolating synthetic cannabinoids from the plant material in these various herbal mixtures (34–48).

In this report the synthetic cannabinoid extraction efficacies of four solvents at two different concentrations, 2% and 100% solvent, are studied. A low-solvent concentration allows for a more environmentally friendly extraction procedure in addition to potentially reducing the level of extracted plant material. Furthermore, a concentration of 2% solvent is compatible with more biomolecules and thus would enable the extracts to be added directly to a biomolecule-based sensing platform. To determine the success of each extraction protocol the quantity of extracted synthetic cannabinoids was considered and the total mass percentage of synthetic cannabinoid extract in comparison to contaminants. Three different incubation periods were used to determine the shortest timeframe necessary to extract the most synthetic cannabinoid. The objective of these studies is to isolate the simplest and most efficient method of extracting synthetic cannabinoids from a synthetic cannabinoid substrate for further forensic investigation.

2. Materials and Methods

2.1 Chemicals

Dimethyl sulfoxide (DMSO), chloroform, methanol, ethanol, acetonitrile, formic acid, potassium chloride, and Win 55, 212-2 were purchased from Sigma Aldrich (St. Louis, MO). All solvents were of HPLC* grade or higher and used without further purification. Ultra pure milli-Q water was used for all experiments. Damiana leaves cut and sifted were purchased from Holistic Herbal Solutions, LLC (Grove City, OH).

2.2 Preparation of Spice-Like Herbal Product

To create a spice-like product for synthetic cannabinoid extraction testing we used the damiana leaf (figure 2A) as a substrate for SC deposition, which is one of the more prevalent substrates used in spice products. The SC Win 55, 212-2 (figure 2B) was dissolved in ethanol and subsequently uniformly deposited through immersion of damiana leaf substrate into the SC solution and subsequent drying. Each extraction trial used 100 mg of the damiana leaf doped with 20 mg of the Win 55,212-2. Afterwards, the doped damiana leaves were allowed to dry at room temperature for approximately 1 h and divided into 10-mg aliquots.

* High-performance liquid chromatography.

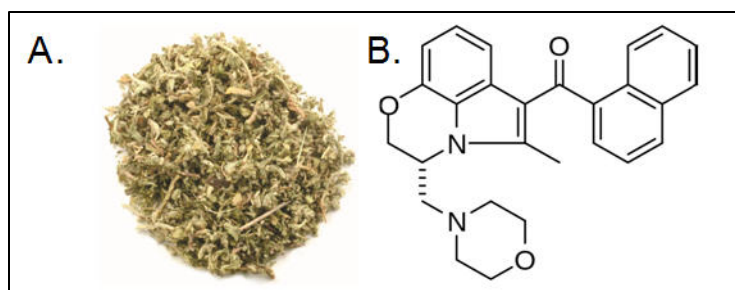


Figure 2. Representative image of damiana leaves (A) and the chemical structure of Win 55, 212-2 (B) used in the study.

2.3 Synthetic Cannabinoid Extraction Studies

All studies were performed in triplicate.

2.3.1 Extraction Solvent Studies

Four different solvents: DMSO, chloroform, methanol, and ethanol, were tested for their ability to extract SCs from doped damiana leaves at 2% solvent concentration and 100% solvent concentration. Each solvent also consists of 0.1 M KCl to allow for an ultimately electrolytic extraction solution. Control extraction experiments were done with 10 mg of damiana leaves while the doped samples contained 10 mg of damiana leaves spiked with approximately 2 mg of Win 55, 212-2. All samples were sonicated with 1 ml of solvent for 30 min. The extract was then isolated from leaf fragments with pipette for further characterization via LC-MS.

2.3.2 Extraction Time Studies

Four different solvents, DMSO, chloroform, methanol, and ethanol, were tested for their ability to extract SCs from doped damiana leaves at two additional timepoints, 30 and 60 s. Each solvent also consists of 0.1 M KCl to allow for an ultimately electrolytic extraction solution. Control extraction experiments were done with 10 mg of damiana leaves while the doped samples contained 10 mg of damiana leaves spiked with approximately 2 mg of Win 55, 212-2. Leaf extracts were prepared via a manual hand shaking method with 1 ml of solvent for either 30 or 60 s, which could be easily used in the field for quick extraction of spice products. The extract was then isolated from leaf fragments with a pipette for further characterization via LC-MS.

2.4 Liquid Chromatography Coupled Mass Spectrometry Analysis of the Damiana Leaf Extracts

The overall purity of the Win55, 212 extracts was analyzed via LC-MS. A single quadrupole Agilent 6130 mass spectrometer was used in conjunction with an Agilent 1200 series LC system (Agilent Technologies, Santa Clara, CA). The LC column was an Agilent Eclipse XDB C18 column (150 × 4.6 mm i.d., 5-μm particle size), maintained at 25 °C with a mobile phase flow rate of 0.6 ml/min. Gradient elution mobile phases consisted of A (0.1% formic acid in water)

and B (0.1% formic acid in acetonitrile) at pH 3.6. The gradient initially began at 30% B and remained isocratic until 2 min. The gradient increased linearly to 50% B from 2 to 6 min and held at that concentration until 12 min at which point the gradient again increased in a linear fashion to 100% B at 26 min. Any remaining compounds were eluted from the column during a wash with 100% B from 26 to 30 min. Detection wavelengths for the LC were 330, 219, and 246 nm as WIN 55, 212-2 has a maximum absorbance at these wavelengths and an expected mass of 427.2. Quantification of the analytes was undertaken using positive scan mode with a molecular mass scan from 100 to 800 g/mol.

3. Results and Discussion

3.1 Characterization of Cannabinoid Extraction Efficiency via LC-MS

Efficient and quick extraction of cannabinoids is critical to successful analysis of suspicious materials in the field. The efficiency of an extraction is determined by two factors; the amount of cannabinoid extracted (signal strength) and the purity of the extract (level of the intrinsic plant compounds in the extract). However, previous studies that developed protocols for the extraction of SCs from leafy substrates have relied heavily on lab-based techniques. These techniques usually require several steps including, grinding up the leafy substrate, sonication, and long extraction times (>10 min) (1, 6, 7, 35, 37, 40, 49). This widely accepted laboratory-based procedure is not advantageous for field use for two major reasons; (1) the use of sonication during solvent incubation and (2) long extraction times. In this comprehensive extraction study we determined the optimal solvent and extraction time in order to develop a facile SC extraction method that could be easily conducted in the field. Four common solvents (DMSO, chloroform, methanol, and ethanol) at two different concentrations (2% and 100%) were assessed for overall extraction efficiency. Furthermore, all solvents studied contained 0.1 M KCl in order to make them electrolytic. Electrolytic solvents are conductive and therefore compatible with any electronic-based sensing platform (50).

WIN 55, 212-2 was chosen as the SC for use in this study as it has been widely characterized in the literature and because it is not scheduled by the DEA and is therefore available for purchase without a permit (32, 41, 45). WIN 55 212-2 has a maximum absorbance at 219, 246, and 330 nm and an expected mass of 427.2. Damiana leaves are one of the most common substrates used in the preparation of “spice” like substances (51). Thus, damiana was used as the substrate in the test extractions. Figure 3 shows a set of plots for the control and Win55, 212-2 doped damiana leaf extracts with 100% ethanol for 30 min. These plots are characteristic of the extraction data obtained for all four solvents. The peak that appears at approximately 3.6 min is the cannabinoid elution peak. Mass spectrometry analysis of the 3.6-min peak show pure WIN 55, 212-2 with very little other contaminants (figure 4).

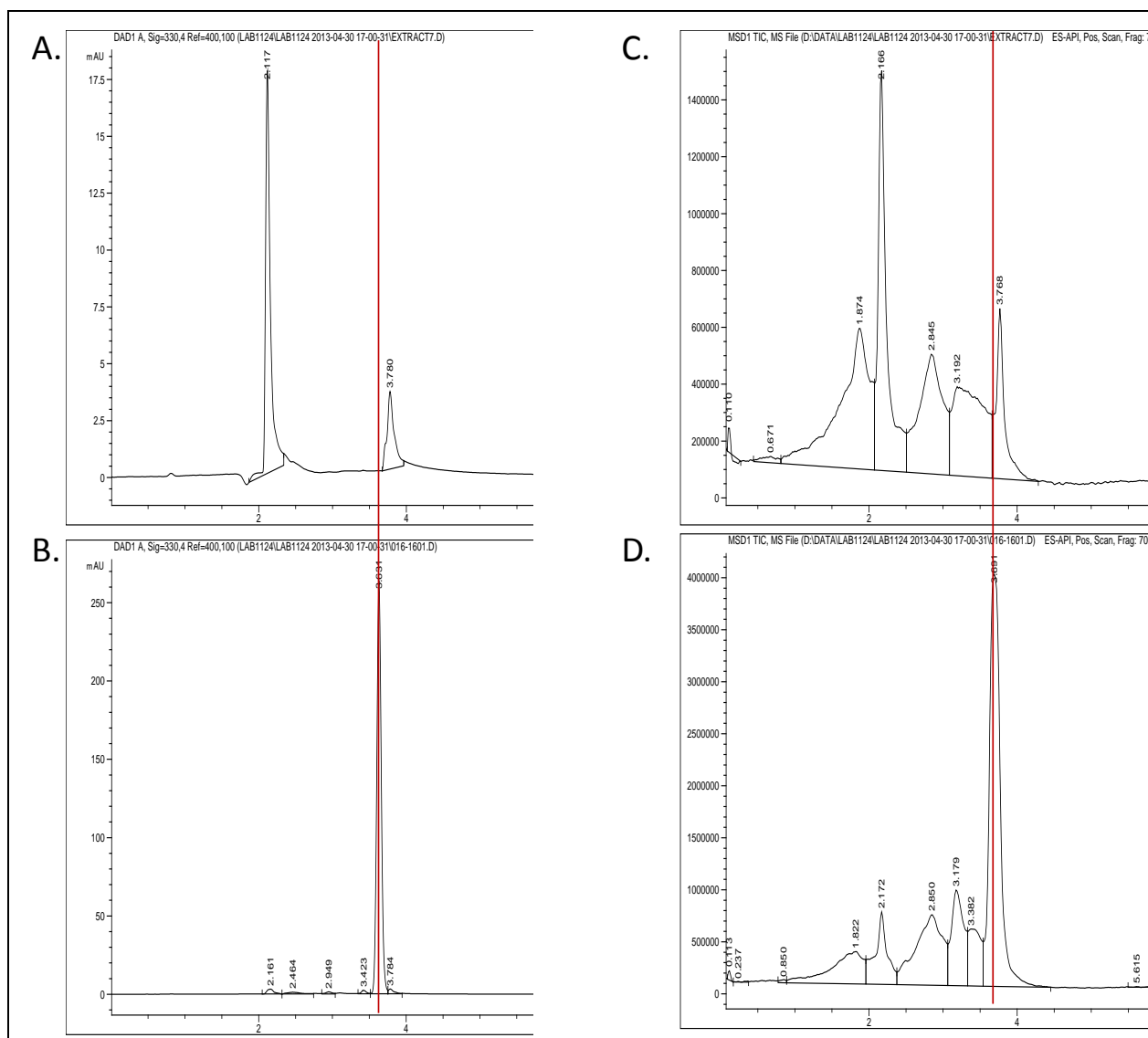


Figure 3. LCMS plots of 100% ethanol extracts of damiana leaves. LC spectra of control (A) and Win55, 212-2 doped (B) damiana leaf extracts monitored at 330 nm. Mass spectral plots of control (C) and Win55, 212-2 doped (D) damiana leaf extracts.

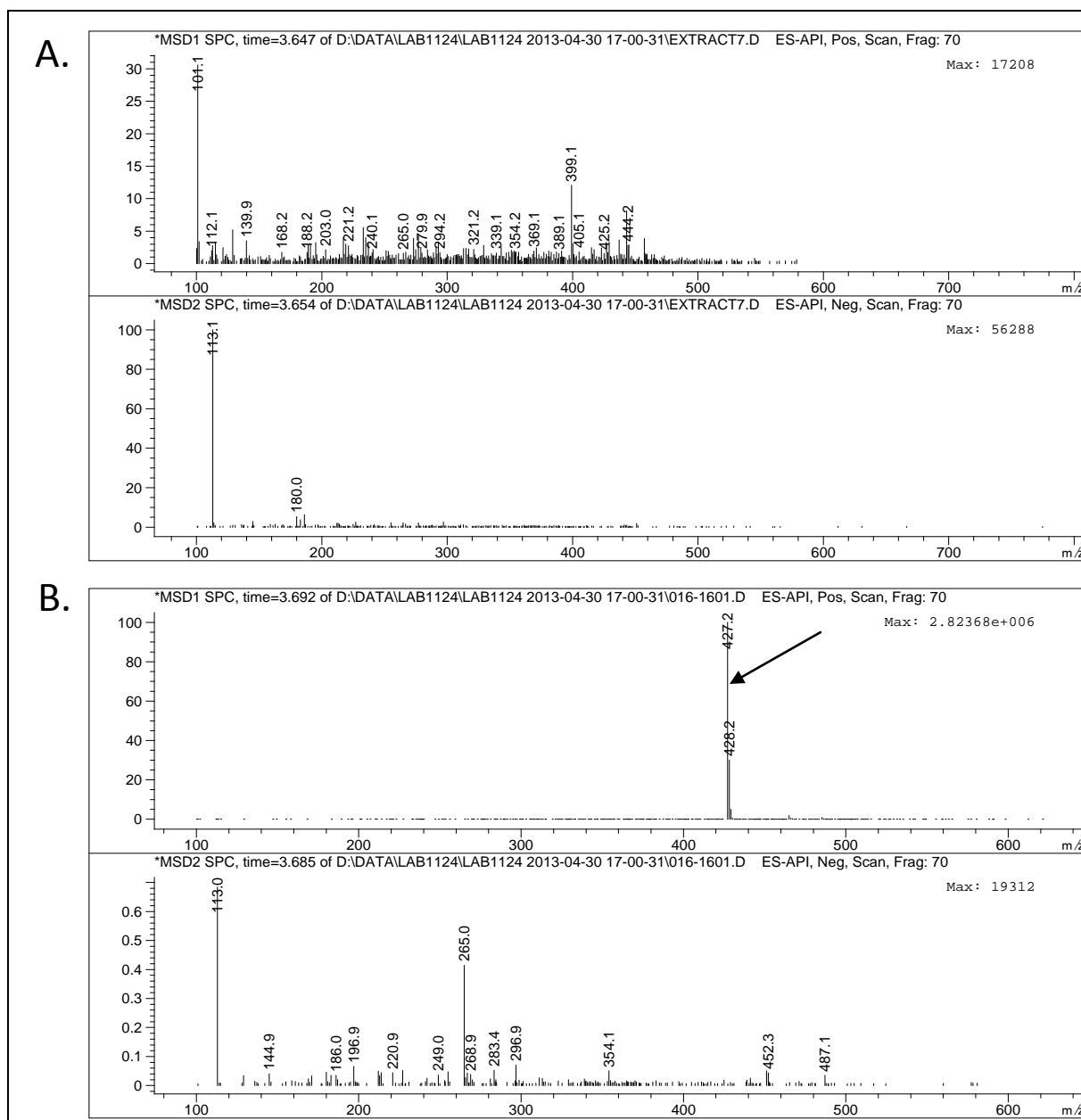


Figure 4. Total extracted mass ion plots at 3.6 min of control (A) and Win 55, 212-2 doped (B) damiana leaf extracts with 100% ethanol. Win55, 212-2 has an expected mass of 427.2 g/mol.

Initially, traditional solvent extraction methods were used to determine if a lower percentage of solvent could be sufficient in cannabinoid extraction. The use of a lower percentage of solvent allows for a more environmentally friendly process with less toxic waste produced. Solvent concentrations of 2% were studied as this concentration is known to be compatible with several biomolecules including the cannabinoid receptor and thus extracts at this solvent concentration could be directly used in any receptor-based detection system. However, the samples with the low-solvent concentration had very low efficiency. The LC chromatogram comparisons between 2% and 100% solvent extractions with the four different solvents are highlighted in figure 5.

Immediately evident is the large increase in Win55, 212-2 yield when 100% solvent is used to prepare the leaf extracts. Peak area and total mass percentage comparisons for the WIN55 212-2 peak demonstrate that the amount of cannabinoid pulled off at 2% solvent is 94 times less than the amount of cannabinoid extracted with 100% solvent (figure 5). As it is only a 50 fold dilution to get from a 100% solvent concentration to a 2% solvent concentration, the more efficient method for extracting SC compounds from leafy substrates would be to extract with 100% solvent and then dilute down to the concentration that is compatible with the assay of interest (i.e., 2% for a receptor-based detection platform).

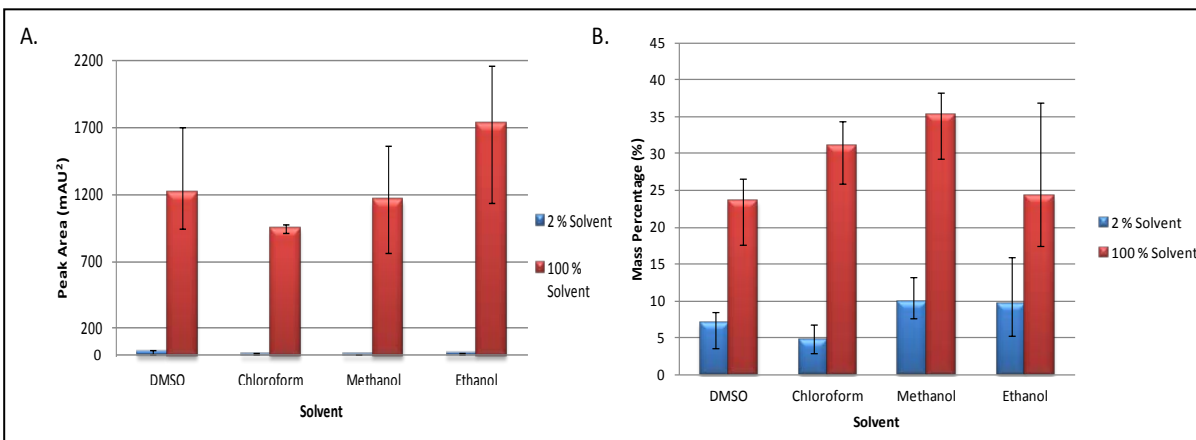


Figure 5. Bar charts of peak area (A) and total mass percentage (B) comparisons of Win 55, 212-2 extraction with 2% (blue) and 100% (red) solvent concentration.

A fieldable extraction protocol must not only provide high quantity and purity of SC compounds post extraction; the total time requirements for the protocol must be low. Thus, it was then necessary to determine the shortest successful extraction time after determination of the ideal solvent concentration. It is not practical to perform an extraction in the field with a sonicator or related agitator instrument; consequently, the preparation of the damiana leaf extracts with hand shaking was chosen. Furthermore, as extraction times greater than a few minutes are also impractical, the study of extraction efficacies at 30 s and 1-min timepoints was chosen. Of importance, the data showed a higher overall mass percentage of WIN55, 212-2 in the extracts for both the 30 s and 1-min extraction times with hand shaking (average 42%) when compared to the 30-min extraction samples that were subject to sonication (average 28%, figures 5 and 6). The higher level of contaminants in the previous study could be attributed to the effects of a longer extraction time coupled with agitation via sonication. In conjunction, these two factors enable a higher amount of inherent damiana leaf compounds to be extracted thus increasing the level of contamination in the Win55, 212-2 extracts. As the second protocol requires agitating for a shorter length of time with a much more gentle method (hand shaking) the only compounds extracted are those that are very easily removed, such as the SCs that are sprayed on the leafy substrates. Notably, the peak area between the two extraction protocols (30-min sonication versus ≤ 1 -min hand shaking) is highly similar with an average peak area of 1259 mAU² for the

former and 1255 mAU² for the latter. These data lend further support to the observation that higher extraction efficiency is achieved with shorter incubation times and gentler agitation methods.

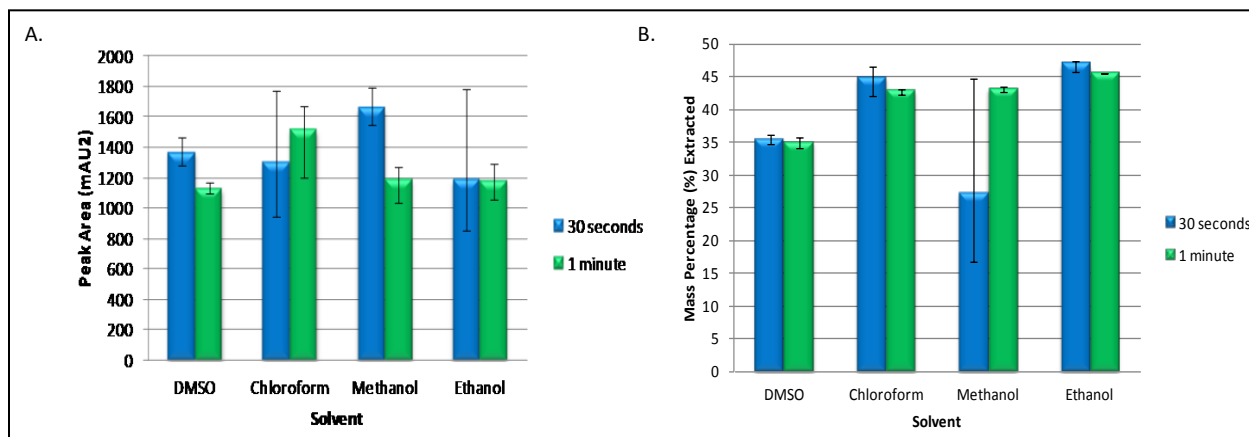


Figure 6. Bar charts of peak area (A) and total mass percentage (B) comparisons of Win 55, 212-2 extraction with 30 s (blue) and 1-min (green) shaking times.

Also of interest was the result that there was no statistically significant difference in the overall extraction efficiency between the 30 s and 1-min timepoints (figure 6). These results show that the easily extracted materials are lifted off leaves almost immediately upon solvent addition. Thus, the more intense extraction methodologies only serve to increase the level of contamination within the extract samples, not increase the amount of SC extracted from the leafy substrates.

4. Summary and Conclusions

In the current study a rapid and facile method to extract SCs from leafy substrates in the field was determined. Win 55, 212-2 was used as the model synthetic cannabinoid while damaina leaf cuttings were used as a representative substrate material. Both low- (2%) and high- (100%) solvent concentrations were assayed for overall extraction efficiency and it was determined that extraction with pure solvent leads to a much higher yield of cannabinoid with ethanol extracting the largest quantity of cannabinoid. Interestingly, there is no significant different between extraction times of 30 s and 1 min with hand shaking. Thus, we have determined that extraction of a small amount of substance (approximately 10 mg) with 100% ethanol and 30 s of shaking will yield a sufficient amount of SC compounds for further analysis techniques. Further studies are needed to determine if these extraction parameters are compatible with both real-world “spice” samples and a wide range of synthetic cannabinoid compounds.

5. References

1. Penn, H. J.; Langman, L. J.; Unold, D.; Shields, J.; Nichols, J. H. Detection of Synthetic Cannabinoids in Herbal Incense Products. *Clin Biochem* September **2011**, *44*, 1163–5.
2. Zuba, D.; Byrska, B. Analysis of the Prevalence and Coexistence of Synthetic Cannabinoids in “Herbal High” Products in Poland. *Forensic Toxicology* January **2013**, *31*, 21–30.
3. Gerona, R. R. L.; Birsdall, E.; Wu, A. H. Non-Targeted Analysis of Synthetic Cannabinoids in Two Intoxication Cases Involving Herbal Incense Products. *American Journal of Clinical Pathology* September **2011**, *136*, 474–475.
4. Langman, L. J.; Penn, H. J.; Unold, D.; Shields, J.; Nichols, J. H. Synthetic Cannabinoids Detected in Select “Herbal Incense” Products. *Therapeutic Drug Monitoring* August **2011**, *33*, 471–471.
5. Uchiyama, N.; Kikura-Hanajiri, R.; Ogata, J.; Goda, Y. Chemical Analysis of Synthetic Cannabinoids as Designer Drugs in Herbal Products. *Forensic Science International* 20 May **2010**, *198*, 31–38.
6. Oyemade, A. Adverse Effects and Detection of Synthetic Cannabinoids. *Am J Addict* November **2012**, *21*, 568–9.
7. Simolka, K.; Lindigkeit, R.; Schiebel, H. M.; Papke, U.; Ernst, L.; Beuerle, T. Analysis of Synthetic Cannabinoids in “Spice-Like” Herbal Highs: Snapshot of the German Market in Summer 2011. *Anal Bioanal Chem* July **2012**, *404*, 157–71.
8. Uchiyama, N.; Kikura-Hanajiri, R.; Ogata, J.; Goda, Y. Chemical Analysis of Synthetic Cannabinoids as Designer Drugs in Herbal Products. *Forensic Sci Int* 20 May **2010**, *198*, 31–8.
9. Hudson, S.; Ramsey, J. The Emergence and Analysis of Synthetic Cannabinoids. *Drug Test Anal.* July-August **2011**, *3*, 466–78.
10. Logan, B. K.; Reinhold, L. E.; Xu, A.; Diamond, F. X. Identification of Synthetic Cannabinoids in Herbal Incense Blends in the United States. *J Forensic Sci.* September **2012**, *57*, 1168–80.
11. Piggee, C. Investigating a Not-So-Natural High Researchers Identify Synthetic Cannabinoids in Herbal Incense. *Analytical Chemistry* 1 May **2009**, *81*, 3205–3207.
12. Camp, N. E. Synthetic Cannabinoids. *J Emerg Nurs* May **2011**, *37*, 292–3.

13. Penders, T.; Saeed, S. Synthetic Cannabinoids and “Bath Salts” Should be Considered Drugs of Abuse. *Am Fam Physician* May 1 **2012**, 85, 852.
14. Ashton, J. C. Synthetic Cannabinoids as Drugs of Abuse. *Curr Drug Abuse Rev.* June **2012**, 5, 158–68.
15. Platteborze, P. L.; Martin, T. M. Re: Spice, Bath Salts, and the U.S. Military: The Emergence of Synthetic Cannabinoid Receptor Agonists and Cathinones in the U.S. Armed Forces. *Mil Med* January **2013**, 178, v.
16. Loeffler, G.; Hurst, D.; Penn, A.; Yung, K. Spice, Bath Salts, and the U.S. Military: The Emergence of Synthetic Cannabinoid Receptor Agonists and Cathinones in the U.S. Armed Forces. *Mil Med.* September **2012**, 177, 1041–8.
17. U. N. O. o. D. a. Crime. Synthetic Cannabinoids in Herbal Products. *U.N. Document* **2011**, 24.
18. McGuinness, T. M.; Newell, D. Risky Recreation: Synthetic Cannabinoids Have Dangerous Effects. *J Psychosoc Nurs Ment Health Serv.* August **2012**, 50, 16–8.
19. Seely, K. A.; Lapoint, J.; Moran, J. H. Fattore, L. Spice Drugs are More Than Harmless Herbal Blends: A review of the Pharmacology and Toxicology of Synthetic Cannabinoids. *Prog Neuropsychopharmacol Biol Psychiatry* 3 December **2012**, 39, 234–43.
20. Ashton, C. H.; Moore, P. B. Regulation of Synthetic Cannabinoids. *Lancet* 7 November **2009**, 374, 1595.
21. Psychoyos, D.; K. Y. Vinod. Marijuana, Spice ‘Herbal High’, and Early Neural Development: Implications for Rescheduling and Legalization, *Drug Testing and Analysis* January **2013**, 5, 27–45.
22. Lindigkeit, R.; Boehme, A.; Eiserloh, I.; Luebbecke, M.; Wiggermann, M.; Ernst, L.; Beuerle, T. Spice: a Never Ending Story? *Forensic Sci Int.* 30 October **2009**, 191, 58–63.
23. King, L. A. Legal Controls on Cannabimimetics: An international dilemma? *Drug Test Anal.* 23 July **2013**.
24. Darmani, N. A. Delta(9)-tetrahydrocannabinol and Synthetic Cannabinoids Prevent Emesis Produced by the Cannabinoid CB(1) Receptor Antagonist/Inverse Agonist SR 141716A. *Neuropsychopharmacology* February **2001**, 24, 198–203.
25. Console-Bram, L.; Marcu, J.; Abood, M. E. Cannabinoid Receptors: Nomenclature and Pharmacological Principles. *Prog. Neuropsychopharmacol Biol Psychiatry* 2 July **2012**, 38, 4–15.

26. Svizenska, I.; Dubovy, P.; Sulcova, A. Cannabinoid Receptors 1 and 2 (CB1 and CB2), Their Distribution, Ligands and Functional Involvement in Nervous System Structures - A short review. *Pharmacology Biochemistry and Behavior*. October **2008**, *90*, 501–511.
27. Grotenhermen, F.; Muller-Vahl, K. The Therapeutic Potential of Cannabis and Cannabinoids. *Deutsches Arzteblatt International* July 23 **2012**, *109*, 495–U39.
28. Hermanns-Clausen, M.; Kneisel, S.; Szabo, B.; Auwarter, V. Acute Toxicity Due to the Confirmed Consumption of Synthetic Cannabinoids: Clinical and Laboratory Findings. *Addiction* 13 September **2012**.
29. Cohen, J.; Morrison, S.; Greenberg, J.; Saidinejad, M. Clinical Presentation of Intoxication Due to Synthetic Cannabinoids. *Pediatrics* April **2012**, *129*, e1064–7.
30. Tomiyama, K.; Funada, M. Cytotoxicity of Synthetic Cannabinoids Found in “Spice” Products: The Role of Cannabinoid Receptors and the Caspase Cascade in the NG 108-15 Cell Line. *Toxicol Lett*. 10 November **2011**, *207*, 12–7.
31. Brakoulias, V. Products Containing Synthetic Cannabinoids and Psychosis. *Aust N Z J Psychiatry* March **2012**, *46*, 281–2.
32. Ginsburg, B. C.; McMahon, L. R.; Sanchez, J. J.; Javors, M. A. Purity of Synthetic Cannabinoids Sold Online for Recreational Use. *J Anal Toxicol* January-February **2012**, *36*, 66–8.
33. Nutt, D. Consideration of the Major Cannabinoid Agonists. *Advisory Council on the Misuse of Drugs Report*, 29, 2009.
34. Kneisel, S.; Auwarter, V.; Kempf, J. Analysis of 30 Synthetic Cannabinoids in Oral Fluid Using Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry. *Drug Test Anal*. 18 October **2012**.
35. Kneisel, S.; Auwarter, V. Analysis of 30 Synthetic Cannabinoids in Serum by Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry After Liquid-Liquid Extraction. *J Mass Spectrom*. July **2012**, *47*, 825–35.
36. Merola, G.; Aturki, Z.; D’Orazio, G.; Gottardo, R.; Macchia, T.; Tagliaro, F.; Fanali, S. Analysis of Synthetic Cannabinoids in Herbal Blends by Means of Nano-Liquid Chromatography. *J Pharm Biomed Anal*. December **2012**, *71*, 45–53.
37. Grabenauer, M.; Krol, W. L.; Wiley, J. L.; Thomas, B. F. Analysis of Synthetic Cannabinoids Using High-Resolution Mass Spectrometry and Mass Defect Filtering: Implications for Nontargeted Screening of Designer Drugs. *Anal Chem*. 3 Jul **2012**, *84*, 5574–81.

38. Ammann, J.; McLaren, J. M.; Gerostamoulos, D.; Beyer, J. Detection and Quantification of New Designer Drugs in Human Blood: Part 1 - Synthetic Cannabinoids. *J Anal Toxicol.* July **2012**, *36*, 372–80.
39. Sobolevsky, T.; Prasolov, I.; Rodchenkov, G. Detection of Urinary Metabolites of AM-2201 and UR-144, Two Novel Synthetic Cannabinoids. *Drug Test Anal.* 5 October **2012**.
40. Hutter, M.; Kneisel, S.; Auwarter, V.; Neukamm, M. A. Determination of 22 Synthetic Cannabinoids in Human Hair by Liquid Chromatography-Tandem Mass Spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 15 August **2012**, *903*, 95–101.
41. Dresen, S.; Kneisel, S.; Weinmann, W.; Zimmermann, R.; Auwarter, V. Development and Validation of a Liquid Chromatography-Tandem Mass Spectrometry Method for the Quantitation of Synthetic Cannabinoids of the Aminoalkylindole Type and Methanandamide in serum and its Application to Forensic Samples. *J Mass Spectrom* February **2011**, *46*, 163–71.
42. Musah, R. A.; Domin, M. A.; Cody, R. B.; Lesiak, A. D.; John Dane, A.; Shepard, J. R. Direct Analysis in Real Time Mass Spectrometry With Collision-Induced Dissociation for Structural Analysis of Synthetic Cannabinoids. *Rapid Commun Mass Spectrom* 15 October **2012**, *26*, 2335–42.
43. Moosmann, B.; Kneisel, S.; Wohlfarth, A.; Brecht, V.; Auwarter, V. A Fast and Inexpensive Procedure for the Isolation of Synthetic Cannabinoids From ‘Spice’ Products Using a Flash Chromatography System. *Anal Bioanal Chem.* 14 October **2012**.
44. Bailey, K.; Legault, D.; Verner, D. Identification of Synthetic Cannabinoids by Gas Chromatography. *J Chromatogr.* 5 December **1973**, *87*, 263–6.
45. Hutter, M.; Broecker, S.; Kneisel, S.; Auwarter, V. Identification of the Major Urinary Metabolites in man of Seven Synthetic Cannabinoids of the Aminoalkylindole Type Present as Adulterants in ‘Herbal Mixtures’ Using LC-MS/MS Techniques. *J Mass Spectrom* January **2012**, *47*, 54–65.
46. de Jager, A. D.; Warner, J. V.; Henman, M.; Ferguson, W.; Hall, A. LC-MS/MS Method for the Quantitation of Metabolites of Eight Commonly-Used Synthetic Cannabinoids in Human Urine--An Australian Perspective. *J Chromatogr B Analyt Technol Biomed Life Sci.* 15 May **2012**, *897*, 22–31.
47. Musah, R. A.; Domin, M. A.; Walling, M. A.; Shepard, J. R. Rapid Identification of Synthetic Cannabinoids in Herbal Samples via Direct Analysis in Real Time Mass Spectrometry,” *Rapid Commun Mass Spectrom* 15 May **2012**, *26*, 1109–14.

48. Cox, A. O.; Daw, R. C.; Mason, M. D.; Grabenauer, M.; Pande, P. G.; Davis, K. H.; Wiley, J. L.; Stout, P. R.; Thomas, B. F.; Huffman, J. W. Use of SPME-HS-GC-MS for the Analysis of Herbal Products Containing Synthetic Cannabinoids. *J Anal Toxicol.* June **2012**, *36*, 293–302.
49. Jankovics, P.; Varadi, A.; Tolgyesi, L.; Lohner, S.; Nemeth-Palotas, J.; Balla, J. Detection and Identification of the New Potential Synthetic Cannabinoids 1-pentyl-3-(2-iodobenzoyl)indole and 1-pentyl-3-(1-adamantoyl)indole in Seized Bulk Powders in Hungary. *Forensic Sci Int.* 10 January **2012**, *214*, 27–32.
50. Wu, Y. C.; Koch, W. F. Absolute Determination of Electrolytic Conductivity for Primary Standard KCl Solutions From 0-Degrees-C to 50-Degrees-C. *Journal of Solution Chemistry* April **1991**, *20*, 391–401.
51. Ogata, J.; Uchiyama, N.; Kikura-Hanajiri, R.; Goda, Y. DNA Sequence Analyses of Blended Herbal Products Including Synthetic Cannabinoids as Designer Drugs. *Forensic Sci Int.* 10 April **2013**, *227*, 33–41.

List of Symbols, Abbreviations, and Acronyms

CB	cannabinoid (receptors)
CB1	cannabinoid receptor 1 (central)
CB2	cannabinoid receptor 2 (peripheral)
DEA	U.S. Drug Enforcement Administration
DMSO	dimethyl sulfoxide
GC-MS	Gas Chromatography Coupled Mass Spectrometry
HPLC	high-performance liquid chromatography
LC-MS	Liquid Chromatography Coupled Mass Spectrometry
SC	synthetic cannabinoid
THC	delta-9-tetrahydrocannabinol

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